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TRANSACTIONS

Oryzanin "Antineuritic Vitamin B". VI.

On the Chemical constitution of "Oryzanin".

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Received April 28, 1937.

In the previous reports(1), the author has assigned the formula $C_{12}H_{16-18}N_4O_2S \cdot 2HCl$ for Oryzanin (B_1) hydrochloride isolated from rice-polishings as well as from yeast. Later studies in 1934 has, however, shown that the analysis agrees better with the formula $C_{12}H_{18}N_4OSCl_2$. The present paper deals with the chemical nature of the cleavage products of Oryzanin.

When the hydrochloride of oryzanin was treated with sodium sulfite according to the procedure of Williams and his colleague(2), two crystalline compounds have been obtained i. e., an acidic sulphonic compound $C_6H_9N_3SO_3$ (II) and a hydrochloride of a base C_6H_9NOS (III).

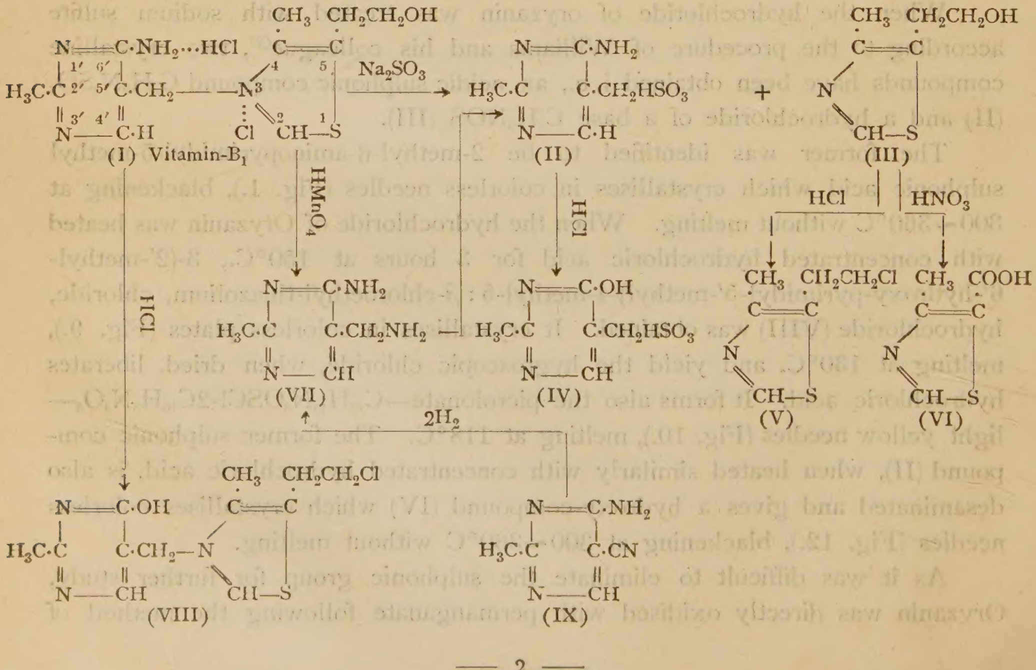
The former was identified to be 2-methyl-6-aminopyrimidyl-5-methyl sulphonic acid which crystallises in colorless needles (Fig. 1.), blackening at 300∼360°C without melting. When the hydrochloride of Oryzanin was heated with concentrated hydrochloric acid for 3 hours at 150°C., 3-(2′-methyl-6′-hydroxy-pyrimidyl-5′-methyl)-4-methyl-5 : β-chloroethyl-thiazolium, chloride, hydrochloride (VIII) was obtained. It crystallises in colorless plates (Fig. 9.), melting at 130°C. and yield the hygroscopic chloride, when dried, liberates hydrochloric acid. It forms also the picrolonate—$C_{12}H_{15}N_3OSCl\text{-}2C_{10}H_8N_4O_5$—light yellow needles (Fig. 10.), melting at 118°C. The former sulphonic compound (II), when heated similarly with concentrated hydrochloric acid, is also desaminated and gives a hydroxy-compound (IV) which crystallises colorless needles (Fig. 12.), blackening at 300∼360°C without melting.

As it was difficult to eliminate the sulphonic group for further study, Oryzanin was directly oxidised with permanganate following the method of

Windaus and his colleague(3), and the hydrochloride of a base $C_6H_{10}N_4$ (VII) has been obtained. The hydrochloride—$C_6H_{10}N_4$-2HCl—crystallises in colorless needles (Fig. 11.) with one molecule of water of crystallisation, melting at 263°C; the picrate—$C_6H_{10}N_4$-$2C_6H_3N_3O_7$—light yellow plates (Fig. 12.), M-pt. 225°C; the picrolonate—$C_6H_{10}O_4$-$2C_6H_{10}N_4O_5$—light yellow prisms (Fig. 13.), M-pt. 250°C; the chlorplatinate—$C_6H_{10}N_4$-H_2PtCl_6—orange yellow plates (VII) (Fig. 14.), blackening at 280~290°C without melting. Thus the above base was proved to be identical with the unknown base [I](4) isolated by the present author from rice-polishings as well as from yeast extract and also with 2-methyl-6-amino-5-aminomethyl-pyrimidin prepared synthetically from 2-methyl-6-amino-5-cyano-pyrimidin (IX) by hydrogenation.

The second cleavaged product of oryzanin was proved to be 4-methyl-5-hydroxyethyl-thiazole (III) existing as the quaternary salt in the molecule of Oryzanin. The hydrochloride of this compound—C_6H_9NOS-HCl—crystallises in hygroscopic colorless prisms (Fig. 3.), M-pt. 95~96°C; the picrate—C_6H_9NOS-$C_6H_3N_3O_7$—light yellow needles (Fig. 4.), M-pt. 164°C; the picrolonate—C_6H_9NOS-$C_{10}H_8N_4O_5$—light yellow needles or prisms (Fig. 5. a, b.), M-pt. 185°C; the chlorplatinate—$(C_6H_4NOS)_2$-H_2PtCl_6—orange yellow prisms (Fig. 6.), M-pt. 173°C; the chloraurate—C_6H_9NOS-$HAuCl_4$—light yellow prisms (Fig. 7.), M-pt. 138°C.

When the hydrochloride of the above base was heated with concentrated hydrochloric acid for 3 hours at 150°C., chloro-compound (V) was obtained. Its hydrochloride—C_6H_8NSCl-HCl forms hygroscopic colorless prisms and its picrate—C_6H_8NSCl-$C_6H_3N_3O_7$—light yellow needles; M-pt. 139°C.



When the methyl-hydroxyethyl-thiazole (III), mentioned above, was warmed with concentrated nitric acid at 60°C, it was oxidised to the known compound 4-methyl-thiazolium-5-carboxylic acid (VI)(5), which forms colorless plates (Fig. 8.) and sublimes at 251°C.

The above results are in full accordance with the observation of Windaus(3) and Williams(2) and furnish further confirmation to the chemical structure of Oryzanin or B_1 proposed by these authors i.e., 3-(2′-methyl-6′-amino-pyrimidyl-5′-methyl)-4-methyl-5 : β-hydroxyethyl-thiazole (I).

The authors wish to express their sincere thanks to Prof. Dr. U. Suzuki for his kind guidance and to Sankyo company Ltd. for kind supply of the material.

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ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

The Influence of Monochromatic Lights on the Action of Yeast Catalase. (Part I.)

(pp. 429～434)

By Reitaro Murakami.

(Agricultural College, Utsunomiya, Received April 6, 1937.)

In order to investigate of spectral monochromatic lights on the action of yeast catalase, the enzyme has been prepared as the refined clear solution by the elution of the adsorption compound with phosphate mixture of 400 cc, which precipitated by the addition of the aluminium hydroxide into the clear extract of the autolysis of "Oriental" pressed yeast of 150 g.

The author has added 1 or 2.5 cc. of enzyme solution into the test tubes or bottles containing respectively 20 or 50 cc of N/20 H_2O_2 solution and the pH of the solution was 6.7.

The test tubes or bottles were placed within the tinned boxes, at the front windors of which the monochromatic filters of red, yellow, green, blue, violet and ultra vit glass were placed. The filters of white and black passing respectively all or no visible rays were also applied as controls. The boxes containing the test tubes or bottles were incubated at 20°C and lighted by an electric lamp through the layer of copper sulphate solution and filters.

The test tubes and bottles were taken out from each box at interval of 15 minutes after the incubation and the undecomposed hydrogen peroxide in the solution was determined by the titration with N/20 K-permanganate solution.

In this experiment, the apparent effect on the yeast catalase was found to be comparatively strongest under the yellow light and stronger under the white light and successively weaker under the rays of red, ultra vit, green, blue and violet. The action was most inferior in the dark. But the difference of the effect between each light was a little.

The proportions of hydrogen peroxide decomposed by catalase for an unit intensity of the irradiated rays and the absorbed rays into the solutions was found to be in inverse effect in comparison with their intensities.

Hence, the rays present the positive stimulant for the yeast catalase but the effect has been decreased succesively with their intensities.

The Influence of Monochromatic Lights on the Action of Yeast Catalase. (Part II.)

(pp. 435～438)

By Reitaro MURAKAMI.

(Agricultural College, Utsunomiya, Received April 6, 1937.)

In order to further investigate the influence of the intensities of spectral monochromatic lights on the action of yeast catalase, the author has continued the research as the previous paper using the monochromatic filters of red, green, blue and violet with respectively possessed of three different intensities.

In this experiment the proportions of hydrogen peroxide decomposed by yeast catalase was found to be increased with the intensities in the same monochromatic light, but to be in inverse effect in comparison with an unit intensity of all monochromatic lights.

It was also found that some rays in spite of the different monochromatic lights present the same effect for an unit intensity. The effect of the rays in such case must be recognized that was caused by the intensities and not by the wave lengths of the lights.

The Influence of Monochromatic Lights on the Action of Soya-Urease. (Part II.)

(pp. 439～443)

By Reitaro MURAKAMI.

(Agricultural College, Utsunomiya, Received April 6, 1937.)

In order to further investigate the influence of the intensities of spectral monochromatic lights on the action of soya-urease, the author has continued the study as the previous paper using the monochromatic filters of red, green, blue and violet which respectively possessed of three different intensities.

The bottles containing 50 cc of 1% urea solution and 5 cc of 0.5% enzyme solution were incubated at 33 °C and lighted by an electric lamp through the layer of copper sulphate solution and the filters.

The bottles were taken out from the incubator at intervals of 2, 6 and 16 hours and the end product, ammonia was determined by PLIMMER and SKELTON method.

In this experiment the proportions of the end product by soya-urease was found to be increased with the intensities in the same monochromatic light, but to be in inverse effect in comparison with an unit intensity of all monochromatic lights.

Hence, it was confirmed that the rays present the positive stimulant action for soya-urease, but the effect has been decreased successively with their intensities.

On the Relation between the Quality of Tobacco and the Content of Pectic Acid and Methylalcohol in it.

(pp. 444~453)

By Shinji KANAHARA.

(Japanese Government Monopoly Bureau, Received April 28, 1937.)

On Unsaponifiable Substances especially Sterol in the Oil of "Tamari."

(pp. 454~457)

By Kenji MIYAJI and Motozo OGURI.

(Agricultural College of Gifu, Japan, Received April 9, 1937.)

Sterilizing Action of Acids. 9th Report.

Relation between the Stereo-chemical Constitution of Fatty Acids isomers and the Physiology of Bacteria. No. II. Optical active acids isomers and physiology of bacteria.

(pp. 458~466)

By Sogo TETSUMOTO.

(Institute for Infectious Diseases, Tokyo Imp. Univ., Received April 13, 1937.)

We see special relation between the formation of optical active acids such as Lactic acid, Malic acid and Tartaric acid, and physiology of micro-organisms. These special relations were studied since 1758th year ago, by Scheele, Pasteur and many other investigaters.

Concerning the influence of d and l polarised light on the growth of Bac. typhosus and Vib. cholerae, Bhatnagar anb Lal's study was reported at the year 1925. But there seems no scientific study concerning the relation between the stereo-chemical constitution of optical active fatty acids such as d, l, r or d, l, r and meso isomers and the physiology of the bacteria.

I performed this experiment to know the special rclation between the stereo-chemical constitution of optical active fatty acids and physiology of bacteria.

Results obtained are summarised as follows.

(1) Optical active fatty acids of stereo-chemical constitution, have the strong action not only on optical active property at physics but also on sterilizing power at physiology of bacteria than optical inactive acids.

(2) Salts of optical inactive acids such as racemic acids of Lactic acid, Malic acid and Tartaric acid or meso Tartaric acids have nearly the same elongative power for surviving period of bacteria.

(3) Optical active fatty acids have not only the physical property but also have the strong activity for bacterial life.

Nutritional Chemistry of the Flowers. (Part I.)

Vitamins and Proteins in Wistaria-Flowers. (Kraunhia floribunda Taub. var. typicà Mak.)

(pp. 467～472)

By Kinsuke KONDO and Sakae SHINANO.

(Laboratory of Nutritional Chemistry, Kyoto Imp. University, Received April 11, 1937.)

(1) The general compositions of the wistaria-flowers were analysed with the result that these flowers contained a pretty amount of the nitrogenous substances.

(2) For the determination of the forms of these nitrogenous substances, some experiments were undertaken. And it was shown that the wistaria-flowers contained some amount of the conjugated protein, lightly combined with carbohydrates and colouring matters.

(3) The wistaria-flowers were used as vitamin-sources in the feeding of albino-rats, and it was proved that these flowers contained the suitable amount of vitamin A, B-complex and E-active substance. Then we may say that this flower contains carotin or kryptoxanthin and flavin as colouring matters. (1937, March 25)

The Colorimetric Method for the Determination of Carbohydrate in Protein-Molecule.

(The modified Sörensen's Method.)

(pp. 473～493)

By Kinsuke KONDO and Masashi MURAYAMA.

(Laboratory of Nutritional Chemistry, Kyoto Imp. University, Received April 24, 1937.)

Recently M. Sörensen discovered that she could not determine the carbohydrate in protein-molecule by her orcinol method, especially when the protein contained the higher amount of the tryptophan and the low amount of the carbohydrate. We have had also the same experiences as M. Sörensen had, but we determined the intensity of the colour by means of our photoelectric photometer (equipped with "Cesium Photoelectric Vacuum Tube") instead of stufenphotometer, which was applied by Sörensen.

In the course of experiments, however, we found that the orcinol, added into the protein-H_2SO_4 solution, did react not only with the carbohydrate in protein-molecule, but also with the protein-constituents, and that the protein-H_2SO_4 solution coloured when it was warmed, even if without containing orcinol. These taught us that there would be mistake in using of the protein-H_2SO_4 solution as the control solution. Herewith, we modified the Sörensen's method and could show that we could determine any carbohydrate in protein-molecule by our modified method.

The summary is shown as follows.

(1) The two filter solutions (green and violet) were prepared and examined that these were suitable for our experiments by means of our photoelectric photometer, and also the another filter solution was composed to remove the heat ray and a part of the infra-red ray.

(2) It was shown that we could not determine qualitatively the carbohydrate in casein by the Sörensen's method. The time-curves obtained of casein did not coincide with those of any known carbohydrate, or any mixture of two or three kinds of carbohydrates. Such an incomprehensible results were obtained also with glutenin, sericin or crab-meat protein. But, respecting the Sörensen's original idea, we endeavoured to modify her method.

(3) The details in modifying the Sörensen's method were explained in five sections as follows.

(a) When a casein was warmed in conc. H_2SO_4(6 : 4), the solution coloured in dark reddish brown, but this colouring was controlled by the presence of orcinol. By this fact it would become clear that we may not eliminate the influence of sulfuric acid on the protein by using the casein-H_2SO_4 solution (contained no orcinol) as a control solution. After numerous experiments, we could find that the catechol (Pyrocatechin) was the compound which we had searched.

(b) Assuming that the principal cause for the colouration of protein in the conc. H_2SO_4-solution would be the presence of tryptophan, we showed that this colouration could be controlled similarly by catechol or by orcinol.

(c) It was shown that the colouration of the protein in the conc. H_2SO_4-solution found the cause not only in the presence of tryptophan, but also in the presence of the other protein-constituents, and that this colouration would be controlled by catechol positively or negatively. And the latter phenomena would be the same even with orcinol. Hereupon, if the control solution contains some amount of the catechol, the protein-effects in the colouration

will be conveniently eliminated. (See (4)).

(d) The amount of orcinol to be added to the reaction-solution was examined and it was determined that 3 cc. of the orcinol solution (2% ; in 1 : 4 H_2SO_4) and 20 cc. of reaction-H_2SO_4 (6 : 4) were to be added to 1 cc. of the protein solution.

(e) Our modified method was described shortly.

(4) It may be true that the carbohydrate in the hen's egg albumin (crystallizable) is mannose. However, if we examine the egg albumin by the Sörensen's original method by means of our photometer, we can not detect the mannose. This may mean that our photometer gives us more delicate results than those given by the stufenphotometer.

However, by our modified method we could plainly determine the mannose in the hen's egg albumin with the amount of 2.19%. This would be, we may believe, the proof for the applicability of our modified method to determine the carbohydrate in any protein-molecule, with the gratified results.

(March 27th, 1937.)

Zuckerrohrwachs. I. Mitteil. Über die Phytosterine von Zuckerrohrwachs.

(ss. 494~501)

Von Tetuo Mitui.
(Aus d. Agrikulturchem. Laboratorium d. Kaiserl. Universität Kyoto,
Eingegangen am 9, 4, 1937.)

Aus dem Presskuchen, einem unbrauchbaren Nebenprodukt bei der Rohrzuckerfabrikation, wurde durch Benzol-Extraktion etwa 8%iges Wachs erhalten. Von diesem Rohmaterial konnte der Verfasser in einer guten Ausbeute (ca. 14% nach Digitonin-Verfahren) Phytosterin gewinnen. Es wurde mittels der üblichen Behandlungen(3) mit Essiganhydrid und Brom-Eisessig in äther Lösung in zwei Komponenten gespalten. Auf Grund eines Vergleiches der Schmelzpunkte der Derivate erwies sich ein Sterin als identisch mit dem *Stigmasterin*, wie folgende Tabelle zeigt.

		Schmelzpunkt (nach d. Verfasser)	Schmelzpunkt (nach d. Literatur)
Stigmasterin		168,5~169	170,(3) 169~170(4)
〃	-acetat	139~140	141,(3) 143,6~144,2(4)
〃	-propionat	119,5~120	122(3)
〃	-benzoat	161~161,5	160(3)
〃	-3,5-dinitrobenzoat	227~228	——
〃	-cinnamat	155	155(2)
〃	-acetat-tetrabromid	204~205	202~203,(4) 205~206,(1) 211~212(3)
〃	-propionat-tetrabromid	202~203	202(3)
〃	-benzoat-tetrabromid	173	——
〃	-chlorid	91~92	95(3)

Das zweite Zuckerrohrsterin gehört zu dem *Sitosterin* (von Schmelzpunkt 137~138), welches nach neueren Befunden(5, 6, 10) keinen einheitlichen Stoff darstellt. Die Schmelzpunktangaben der Derivate dieses Sterins sind:

	Schmelzpunkt (nach d. Verfasser)	Schmelzpunkt (nach d. Literatur)
Sitosterin	137~138	137~138(3, 8, 9)
〃 -acetat	134~135	127~128(8)
〃 -propionat	113	108(8)
〃 -benzoat	141~142	145,5(8, 9)
〃 -3,5-dinitrobenzoat	200~201	199~200(7)
〃 -cinnamat	152,5~153	158(8)

Die Ausbeute an Stigmasterin sowie an Sitosterin beträgt 0,14% bzw. 0,77% des Presskuchens.

Wie man aus diesen Prozentzahlen leicht ersehen kann, *ist das Zuckerrohrwachs ein zur Stigmasterin-Gewinnung sehr geeigneter Naturstoff.*

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Physiologically Active Crystalline Esters of Vitamin A. (Part II.)

(pp. 502~506)

By Sadayuki HAMANO.

Über die Verschiedene Eigenschaften von Aspergillusarten. (V).

(ss. 507~)

von Y. TAKEDA u. O. TAKEUTI.

(The Department of Industry, Government Research Institute, Taiwan, Japanese, Received April 24, 1937.)